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Confirmation of a wear-compensation mechanism in dental roots of ruminants

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Abstract

Diet affects many factors of an animal's anatomy, but teeth are a specific focus of dietary research, as their durability lends them to record information on a large variety of scales. Abrasive diets like those of grazing herbivores are known to wear down teeth, but how that wear affects tooth growth and the relations between its different morphological components is rarely investigated. Seven pelleted diets varying in abrasive size and concentration were fed over a 17-month period to 49 sheep (*Ovis aries*), of which $n = 39$ qualified for morphology measurements. Using computed tomography, scans of the skulls were made over the course of the experiment, and the impact of diet-related wear was observed on tooth volume and morphology, including the position of dental burr marks, over time. Digital caliper measurements were applied to 3D renderings of the teeth, and the volume of crown and root segments were investigated separately. We aimed to detect a signal of root growth compensating for wear, and test if this mechanism would be affected by dietary abrasives. Crown-segment volume loss was correlated to root-segment volume gain. Height and burr mark measurements indicated a much higher experimental tooth wear than that previously reported for free-ranging animals. The reason for this is unclear. There was no relationship between tooth height and dentine basin depth. For all parameters, there was no effect of diet; hence, while the measurements corroborate general understanding of tooth wear and compensatory processes, these methods appear not suitable to assess subtle differences between feeding regimes.

KEYWORDS
3D imaging, dietary signal, incipient hypsodonty, root growth, ruminant, tooth volume, tooth wear

1 | INTRODUCTION

Mammalian teeth are mainly composed of layers of mineralized tissue. Generally, the tooth's inner pulp provides innervation and vascularization (Ungar, 2010) and is covered by a thick layer of dentine, itself covered by a hard enamel surface in the crown, and by cementum in the roots. Cementum is an avascular mineralized tissue belonging to the periodontium, or dental attachment apparatus, and mainly serves to anchor the periodontal ligament to the root surface (Gonçalves et al., 2005). Cementum layers are deposited continuously throughout life (Zander & Hürzeler, 1958) and have been used in hypsodont animals to determine an animal's age based on the number of cementum rings in the roots of its teeth (Klevezal, 1996), as these rings are not subject to resorption. Klevezal (1996) also notes that the volume of these cemental layers may be triggered by the amount of mechanic stress to which the tooth is subjected, as some of the thickest layers are seen in beaver, for example, (*Castor fiber* and *Castor canadensis*).

From a developmental viewpoint, hypsodont or high-crowned teeth are achieved by delaying root formation during ontogeny. The differing levels of hypsodonty thus depend on the timing of the crown-root transition, triggered by an expression of different growth factors regulating stem cell transformation and marking the end of the crown morphogenesis (Renvoisé & Michon, 2014). Euhypsodont animals, most often rodents, have ever-growing teeth with open apices (Janis & Fortelius, 1988), as their crown-elongation phase lasts for the majority of the animal's lifetime, continuously delaying root formation (von Koenigswald, 2011). In hypsodont ungulates such as horses, roots start to form extremely late in their ontogeny (Semprebon, Rivals, & Janis, 2019). This happens to a lesser extent in some artiodactyls (e.g., *Antilocapra americana*, *Capra hircus*, and *Ovis aries*), and has been called incipient hypselodonty (Aiglstorfer & Semprebon, 2019; Webb, Hulbert, & Richard, 1986; Witzel, Kierdorf, Frölich, & Kierdorf, 2018).

Though studies in rodents have investigated some aspects of the genetic regulation (Koehne et al., 2016; Tummers & Thesleff, 2003, 2008) and the cellular components (Sonoyama, Seo, Yamaza, & Shi, 2007) of root growth and cementogenesis, the full scope of cementum apposition still unclear and virtually unknown in species outside of these model organisms. In an experiment on goats (*C. hircus*), harder foods impacted cementum production, which was microscopically translated by a reorganization of Sharpey's fibres in the cementum microstructure as a response to increasing tensile forces (Lieberman, 1993). This reaction could be indicative of a feedback mechanism compensating for tooth wear in hypsodont animals, a mechanism discussed in the literature, but which has yet to be explored in detail. Cementum growth compensating for tooth wear has been hypothesized (Attwell, 1980; Klevezal, 1996; Renvoisé & Michon, 2014) and shown experimentally in our pilot study on goats (Ackermans et al., 2019). During this pilot study, volumetric measurements of molar teeth showed a small but consistent gain in root volume when the crown was worn down over time, and the highest wear, caused by high-abrasion diets, was correlated to the highest volume gain in the root segment.

As a follow-up of the goat pilot study, the present study consists in a larger feeding experiment, lasting three times as long, in which sheep (*O. aries*) were fed pelleted diets of different abrasive concentration and size. The aim of the present study was to test whether a similar signal of root growth compensating for wear could be detected in the sheep, and if this mechanism would be affected by a variety of concentrations and sizes of abrasives. As in the goat pilot study, we performed volume measurements on the crown and root segments of the molar M2, and additionally compared digital caliper measurements on 3D renderings of the teeth from the beginning and end of the experiment, as a simple method to evaluate morphometric changes. We also evaluated the use of burr marks applied to the sheep's molars as a tool to measure changes in the wear and growth of the teeth. Burr marks have been used successfully in determining wear and growth rates in hypselodont teeth (Meredith, Prebble, & Shaw, 2015; Müller et al., 2014, 2015), but to our knowledge, have never been applied in non-hypselodont molars. Finally, we quantified the depth of the occlusal dentine basins. Recent work has found a consistent, negative correlation between dentine basin depth and overall tooth height in a rodent with hypseledont cheek teeth Martin et al. (2019), but no similar relationship in a ruminant (Sanson, Kerr, & Read, 2017), and we aimed at corroborating the latter finding in our sample.

2 | MATERIAL AND METHODS

2.1 | Experimental animals and diets

The animal experiment on which the present study is based is the same as in Ackermans et al. (2019). The experiment was performed with approval of the Swiss Cantonal Animal Care and Use Committee Zurich (animal experiment license no. 10/2016). Forty-eight ewes and one wether (*O. aries*, $n = 49$, wether: ZMH109537) of mixed breeds (Braunköpfiges Fleischschaf, Dorper, Ile-de-France Suisse, Oxford, Schwarzbraunes Bergschaf, Skudde, Spiegelschaf, Texel, and Weissen Alpenschaf; average body mass = 75 ± 16 kg) and varying ages (estimated age = 3–10 years, exact ages unknown—all had third molars that were erupted, and in use, as evaluated during initial computed tomography [CT]) were part of this study. The animals were divided into seven groups and fed pelleted diets of varying abrasiveness. Two concentrations (4 and 8%) and three sizes (s: ⌀ 4 μm, m: ⌀ 50 μm, and l: ⌀ 130 μm) of quartz abrasives were added to a lucerne-based pelleted diet to create a total of seven diets, including a control with no abrasives (C: 4%s, 8%s, 4%m, 8%m, 4%l, 8%l). All diets were formulated to be isocaloric and isonitrogenic, using an indigestible nonsilacious filler; a complete description of the diets is reported in Ackermans, Martin, et al. (2019). Ten animals were euthanized due to health complications unrelated to the experiment goal, leaving a total of $n = 39$ animals for the final evaluation. The animals were kept for 17 months, from December 2016 to May 2018, and group-housed on soil or sawdust bedding with a concrete outdoor space (total surface of ca. 15 m^2 per group of eight; the minimum requirement according to the Swiss Animal Welfare Ordinance for eight sheep ≥90 kg is 12 m^2). The animals were also provided with enrichment and salt blocks as a supplement. Water was offered for ad libitum consumption.

CT scans were obtained under general anesthesia at two time points during the experiment (T1: December 2016, T2: April 2017) and on the skull of the animals, postmortem after the experiment (T3: May 2018). Then the skulls were rendered in 3D for volumetric calculations and digital caliper measurements on the teeth (Figure 1). At the time of the first CT, while animals were under anesthesia, burr marks were made on the buccal side of the maxillary second molar teeth using a dental drill, taking care not to drill through the enamel completely. When these marks were visible on the CT (Figure 2), they were used to track tooth wear by measuring this point in relation to the crown tip, alveolar socket, and root tips (Table 1, Figure 1). Because several of these burr marks had worn off by the time of the second CT, evaluations regarding burr marks could only be done on a limited number of animals, and only between T1 and T2.

At the end of the experiment the animals were anesthetized with 0.5 mg/kg body weight of xylazine (*xylazinum*, Xylazin Streuli ad us vet; Streuli Pharma AG, Uznach, Switzerland), and 5 mg/kg body weight of ketamine (Ketanarkon 100 ad us vet; Streuli Pharma AG) intramuscularly, and euthanized with 100–150 mg/kg body weight of pentobarbital (*pentobarbitalum natricum*, Esconarkon ad us vet; Streuli Pharma AG; 100–150 mg/kg body weight) intravenously, and the death of the animals was confirmed by heartbeat reversal. The skulls were prepared by enzymatic maceration at the Center of Natural History, University of Hamburg, where they are permanently curated in the Mammals Collection.

2.2 | CT imaging

The CT methodology is as in Ackermans et al. (2018). Images were acquired from a helical multislice Siemens scanner (Siemens Medical Solutions, Erlangen, Germany).

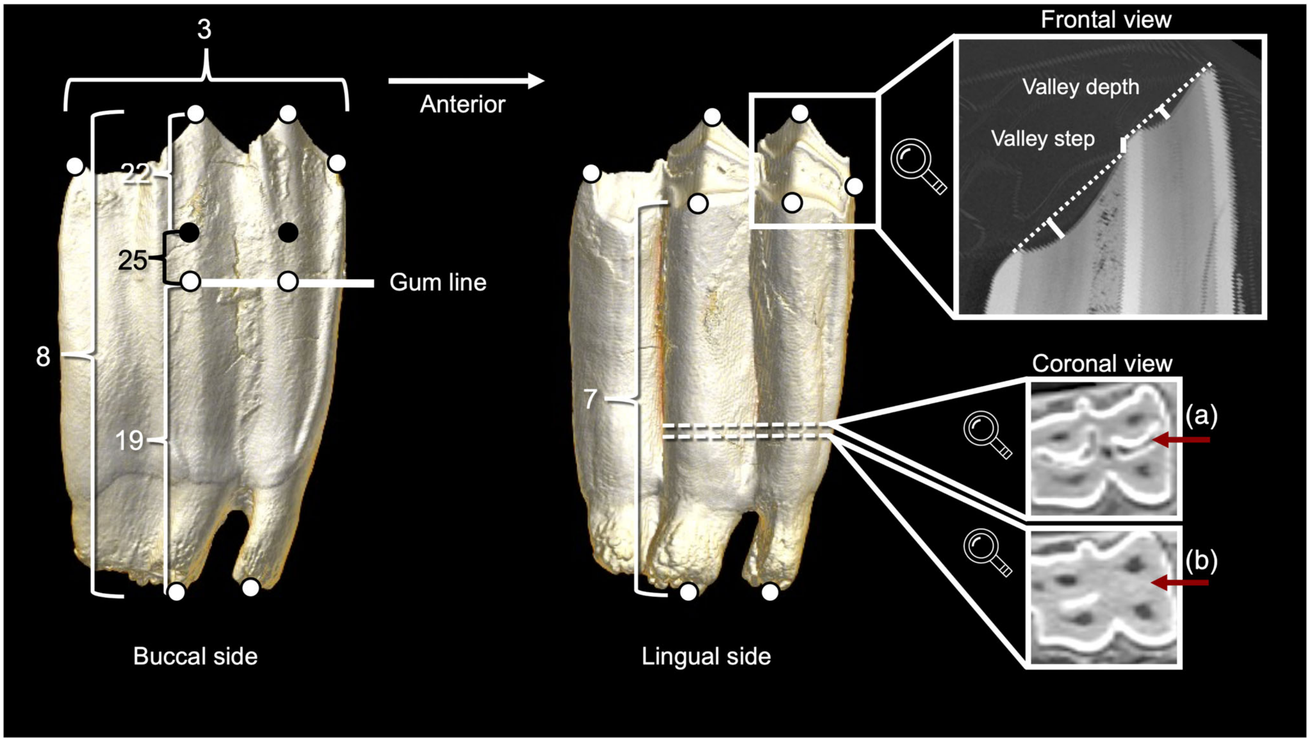

FIGURE 1 Landmarks for digital caliper measurements applied to the teeth of experimental sheep (*Ovis aries*). The tooth represented here is the left mandibular M3 of a goat (ZMH10881), though the measurements were performed on a right mandibular M2. White points indicate points between which digital caliper measurements were made, listed in Table 1, black points indicate burr marks. Panels (a) and (b) indicate computed tomographic (CT) sections visualizing the landmark (red arrow) used to section the tooth into crown and roots. Panel (a) represents the section closest to the crown, while Panel (b) represents the section closest to the roots, where the infundibula disappear. Micro-CT image courtesy of the Institute of Anthropology at the University of Zurich

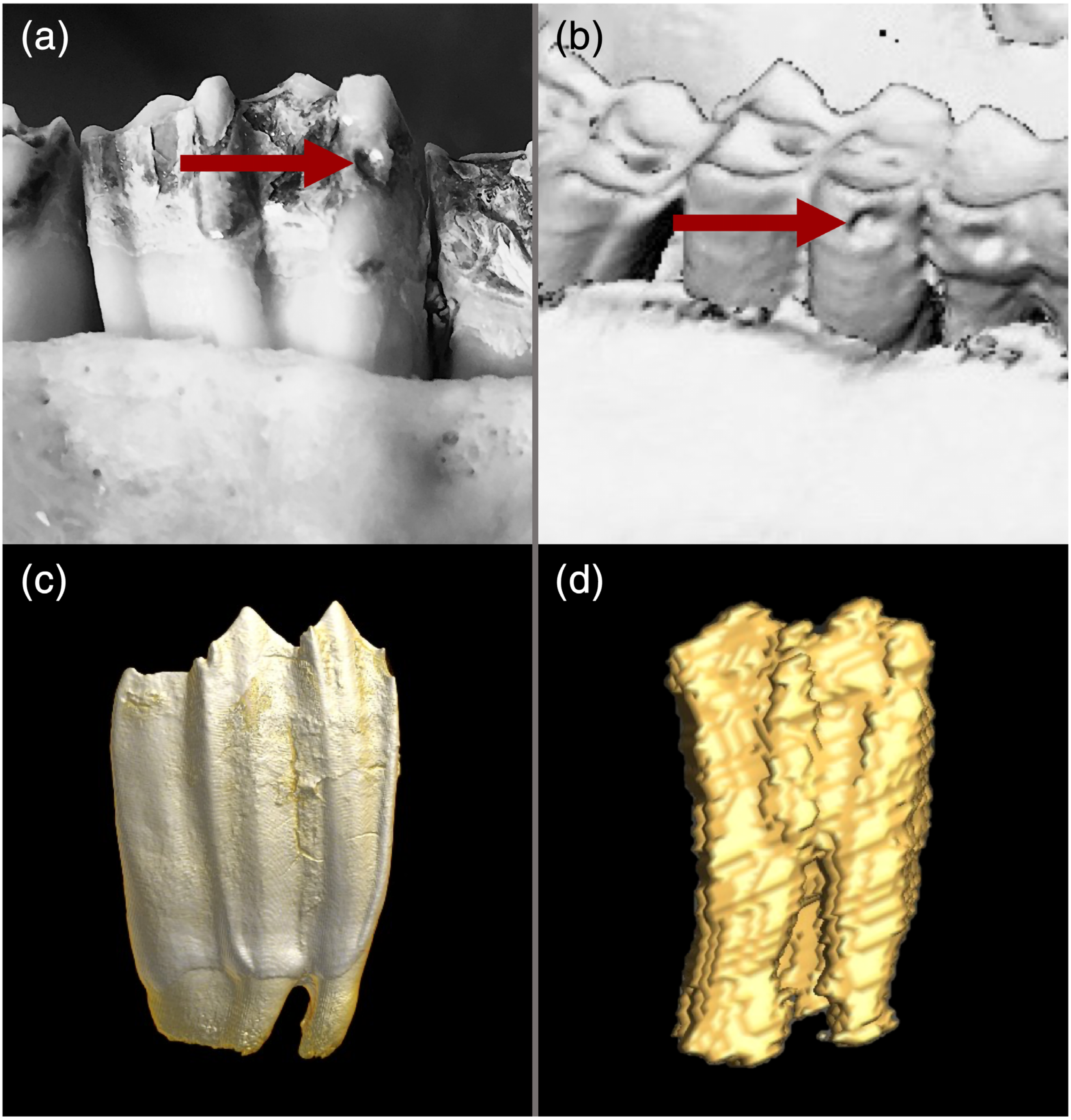

FIGURE 2 Visualization of sheep (*Ovis aries*) teeth with different media. (a) Image and (b) 3D rendering from a medical computed tomographic (CT) scan of a second molar. Burr marks are indicated by arrows. (c) Micro-CT scan of a third molar and (d) a second molar rendered by segmentation from medical CT images

Throughout the experiment, parameters were kept constant: tube voltage at 120 kVp, image matrix of 512 × 512 pixels, field of view of 980 × 332 pixels, slice thickness of 0.6 mm and B60s convolution kernel. During scans, the animals were placed under general anesthesia with ketamine 10 mg/kg (Ketonarkon®, Streuli Pharma AG, Uznach, Switzerland) and xylazine 0.1 mg/kg (Xylazin Streuli, Streuli Pharma AG, Uznach, Switzerland) intramuscularly. Anesthesia was maintained with isoflurane (Attane®, Provet AG, Lyssach, Switzerland) administered in oxygen using a facemask. The first CT scan (T1) was used as baseline for the tooth condition at the start of the experiment, as the animals were in groups but not yet on experimental diets. The CT data sets were converted to DICOM medical imaging format and rendered into 3D surface models using Amira 5.6 (Mercury

TABLE 1 Digital calliper and volumetric measurements applied to the teeth of experimental sheep (*Ovis aries*, n = 39). All measurements were made in millimetres with either a linear measurement tool (L) or a traced, with a non-linear measurement tool (T), volume was measured in µm^3 (V) by semi-automatic segmentation.

#	Measurement	Description	Tool
1	crown volume	volume of the crown from tip until crown/root delimitation	V
2	root volume	volume of the roots from the tip to the crown/root delimitation	V
3	tooth surface length	craniocaudal length of the tooth surface	L
4	height buccal ant	tooth height from the tip of the crown to the tip of the roots on the buccal side of the anterior cusp	T
5	height buccal post	tooth height from the tip of the crown to the tip of the roots on the buccal side of the posterior cusp	T
6	height lingual ant	tooth height from the tip of the crown to the tip of the roots on the lingual side of the anterior cusp	T
7	height lingual post	tooth height from the tip of the crown to the tip of the roots on the lingual side of the posterior cusp	T
8	mean tooth height	mean height calculated from the 4 height measurements per tooth	
9	valley buccal ant	depth of the crown surface valley on the buccal side of the anterior cusp	L
10	valley lingual ant	depth of the crown surface valley on the lingual side of the anterior cusp	L
11	valley buccal post	depth of the crown surface valley on the buccal side of the posterior cusp	L
12	valley lingual post	depth of the crown surface valley on the lingual side of the posterior cusp	L
13	mean valley depth	mean valley depth calculated from the 4 valley measurements per tooth	
14	valley step ant	depth of the step between both valleys on the anterior cusp	L
15	valley step post	depth of the step between both valleys on the posterior cusp	L
16	mean step height	mean valley step height calculated from the 2 valley step measurements	
17	burr to roots ant	measurement from burr mark to tip of roots for the anterior cusp	T
18	burr to roots post	measurement from burr mark to tip of roots for the posterior cusp	T
19	mean burr-root distance	mean burr-root distance calculated from the 2 burr-root measurements	
20	burr to crown ant	measurement from burr mark to tip of crown for the anterior cusp	T
21	burr to crown post	measurement from burr mark to tip of crown for the posterior cusp	T
22	mean burr-crown distance	mean burr-crown distance calculated from the 2 burr-crown measurements	
23	burr to gum ant	measurement from burr mark to alveolar margin for the anterior cusp	T
24	burr to gum post	measurement from burr mark to alveolar margin for the posterior cusp	T
25	mean burr-gum distance	mean burr-alveolar margin distance calculated from the 2 burr-alveolar margin measurements	

Computer Systems/3D Viz group, San Diego, CA) as well as Horos v3.0.1 (Horos Project 2015) for additional visualization.

To allow for mesowear scoring of the 3D renderings in Amira, dynamic models of the data were rendered by defining a fixed isosurface threshold to achieve the highest bone resolution while avoiding artifacts, and view mode was set to orthographic. Excluding missing or otherwise damaged teeth, as well as CT artifacts, data analysis could be carried out with a group of $n = 31$ individuals for volume and $n = 38$ for some digital caliper measurements; with respect to the visibility of the original burr marks at T2, this number was even more reduced to $n = 18$ individuals.

To allow for digital caliper measurements to be taken on the CT scans in Horos, 3D MPR (Multi-planar reconstruction) mode was selected. The slices were focused on the right maxillary M2 and the following measurements were taken using the "length" tool for linear measurements and the "opened polygon" tool for traced measurements. Digital caliper measurements involving burr marks were made for T1 and T2. Crown and root volume measurements were made for T1 and T3, based on a modified version of the method established in the pilot experiment (Ackermans, Clauss, et al., 2019), that is, when moving from the crown to the roots through the image stack, the disappearance of the bottom margin of the anterior infundibulum, and hence also the disappearance of the central structure separating the two infundibula, was used as a landmark to establish a horizontal plane sectioning the tooth into two parts. Though this segmentation does not represent the exact delimitation between the different layers of crown and root tissue, we define them as a crown and a root segment for simplicity (Figure 1a,b). The tooth pulp was included as part of the entire tooth volume, to prevent changes in pulp cavity volume from affecting the functional measurements of the study. Then, semiautomatic sectioning was applied to segment each volume.

On each right maxillary M2, tooth surface length, tooth height, depth of dentine valleys, height of the step between valleys, and the distance of the burr mark to the crown, gumline (defined as the margin of the alveolar socket on the CT), and roots were measured, to account for tooth movement within the alveolar socket and wear over the course of the experiment (Table 1, Figure 1). Individual caliper measurements were made for each tooth side and cusp on 3D renderings of the CT scans from T1, T2, and T3, when applicable. The measurements were averaged for comparisons in the case of the four "height" measurements (crown tip to root tip on the buccal and lingual sides of the anterior and posterior cusps), the four "valley" measurements (the depth of the dentine valley on the buccal and lingual sides of the anterior and posterior cusps), and "step" measurements (the step created by the infundibulum between the buccal and lingual dentine valley on the anterior and posterior cusps).

2.3 | Statistical analysis

Annual growth and wear rates were calculated by dividing volume changes by the time period between the start and the end measurements and scaling the corresponding value ("per day") up to 1 year by multiplying with 365. Note that this calculation assumes a linear change with time. While we cannot assess whether assuming linearity is actually correct, this step was taken to achieve comparability to other measurements reported in the literature, which are typically given on an annual basis (Damuth & Janis, 2014).

In the first step of data evaluation, measurements taken at the first and last time points for the same parameter were plotted against each other to assess whether data showed a general directionality. In particular, we predicted some measurements to become smaller over time (crown segment volume, tooth height, burr–crown distance), some measurements to become larger over time (root segment volume, burr–root distance, burr–gumline distance), and some measurements to show no systematic change (tooth length, valley depth, step height). These predictions were tested by paired Wilcoxon test. Secondly, correlations were assessed by Spearman's correlation analysis. Regardless of whether some data indicated a normal distribution, the generally small sample size and an aim for consistency in the statistical approach suggested that nonparametric tests be used throughout. Tests occurred between crown segment volume loss and root segment volume gain, between "crown wear" and "root growth" as measured by burr marks, and between the tooth or step height and the valley depth. Due to the generally low sample size per diet, and the lack of an evident pattern in the graphical representations, we did not test for an effect of diet. Analyses were performed using SPSS 25.0 (IBM, Armonk, NY), with the significance level set to .05.

3 | RESULTS

When results were averaged across all specimens, there was a systematic decrease in crown segment volume, tooth height, tooth length, and burr-to-crown distance over time (Table 2, Figure 3a,c,g). By contrast, root segment volume increased significantly over the course of the experiment (Table 2, Figure 3b). For measures of valley depth, step height, burr to alveolar margin, and burr-to-root distances, no significant changes over time appeared (Table 2, Figure 5a–c). Crown segment volume loss and root segment volume gain were significantly correlated (Figure 4a, Table 3), although the effect was not as strong as previously documented in goats (Figure 4b). However, when the crown height loss and root height gain was calculated from burr mark measurements, they did not

TABLE 2 Nonparametric correlations between measurements on teeth of sheep (*Ovis aries*) fed diets of varying abrasiveness for 17 months.

y–x	*n*	*R*	*p*
Root volume gain–crown volume loss	31	.55	**.001**
Burr mark growth–burr mark wear	18	−.23	.345
Step height–tooth height	38	.48	**.002**
Valley depth–tooth height	38	.26	.119
Valley depth–step height	38	.44	**.006**

Note: See Table S1 and Figure 1 for a definition of measurements. Bold values are statistically significant show a systematic relationship (Figure 3, Table 3). The caliper measurements generally indicated that higher teeth had higher steps between the basin valleys (Figure 5a, Table 3), but they did not show a correlation to valley depth (Figure 5b, Table 3). Nevertheless, teeth with higher steps had deeper valleys (Figure 5c, Table 3).

4 | DISCUSSION

It has been previously suggested that herbivores with hypsodont (but not hypselodont) teeth, undergo a process of "continuous eruption" consisting of enamel and dentine formation after tooth eruption (Witzel et al., 2018).

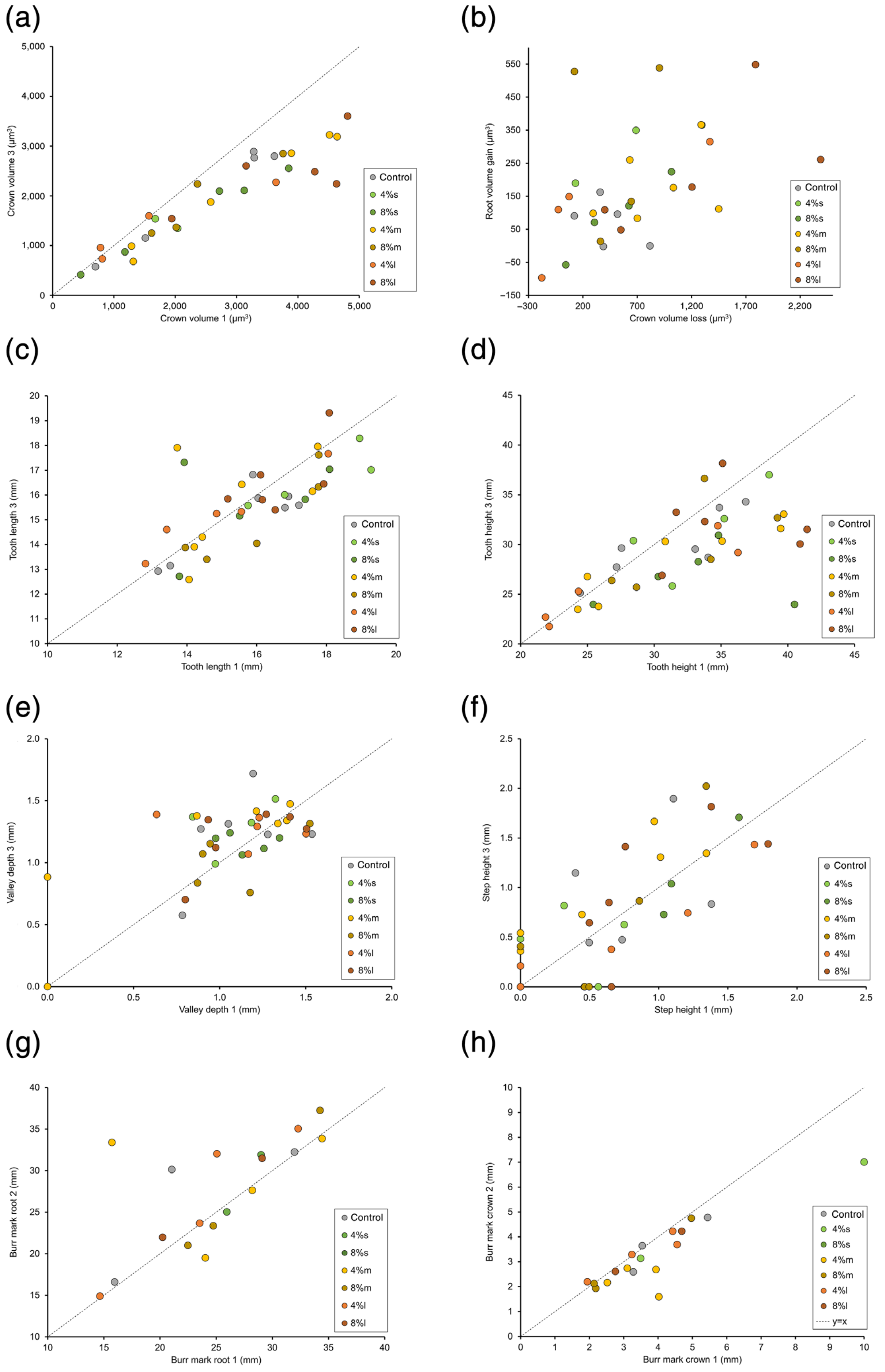

FIGURE 3 Comparisons of measurements made at the beginning (*x* axis) and end of the observation period (*y* axis), measured on teeth of sheep (*Ovis aries*) fed diets of varying abrasiveness for 17 months. The experimental diets include a control diet without added abrasives, two diets containing small abrasives (⌀ 4 µm) at two different concentrations 4%s and 8%s, and the same with both the medium and large abrasives (⌀ 50 and 130 µm). For statistics, see Table 3. (a) Crown segment volume at the beginning of the experiment plotted against crown segment volume at the end of the experiment. (b) The difference in volume of the crown segment plotted in the difference in volume of the root segment. (c) Tooth length at the beginning of the experiment plotted against tooth length at the end of the experiment. (d) Tooth height at the beginning of the experiment plotted against tooth height at the end of the experiment. (e) Average depth of the valleys (dentine basin) at the beginning of the experiment plotted against valley depth at the end of the experiment. (f) Average step height between the two molar cusps at the beginning of the experiment plotted against step height at the end of the experiment. (g) Distance of the burr mark to the roots at the beginning of the experiment plotted against the burr-to-roots distance at the midpoint of the experiment. (h) Distance of the burr mark to the crown at the beginning of the experiment plotted against the burr-to-crown distance at the midpoint of the experiment

This process may continue up to a year after tooth eruption (Balasse, 2002) and emphasizes that the tooth is a live organ rather than an immutable entity. During this phase, cementum apposition is necessary to secure the tooth into the mandibular alveola, which is facilitated by the accretion of cementum tissue directly on the root surface, in acellular and cellular layers that attach to the alveolar bone via periodontal ligaments (Berkovitz & Shellis, 2018). These layers are well known for their fixation properties, though they have been seldom investigated in relation to wear compensation. By microscopically measuring cementum thickness in wild buffalo (*Syncerus caffer*), Sanson et al. (2017) recorded a significant negative correlation between crown height and cementum thickness, also corroborating the existence of a compensatory mechanism. Seemingly, hypsodont teeth continuously adapt during an animal's lifetime, controlling cementum apposition as a reaction to crown wear, as demonstrated by Sanson et al. (2017), our pilot experiment on goats (Ackermans, Clauss, et al., 2019), and the present study.

A comparison of cellular mechanisms involved in the compensatory reaction of hypsodont and hypselodont teeth appears a promising area of future research. In hypselodont teeth, stem cells basal to the tooth's open apex form a structure called the cervical loop (Tummers & Thesleff, 2009), an organ regulating enamel production. Basal stem cells in Hertwig's epithelial root sheath differentiate into odontoblasts and ameloblasts, producing enamel and dentine (Hu, Mushegyan, & Klein, 2014; Thesleff, 2018). In hypsodont teeth, basal cells, possibly originating from the dental follicle (Zeichner-

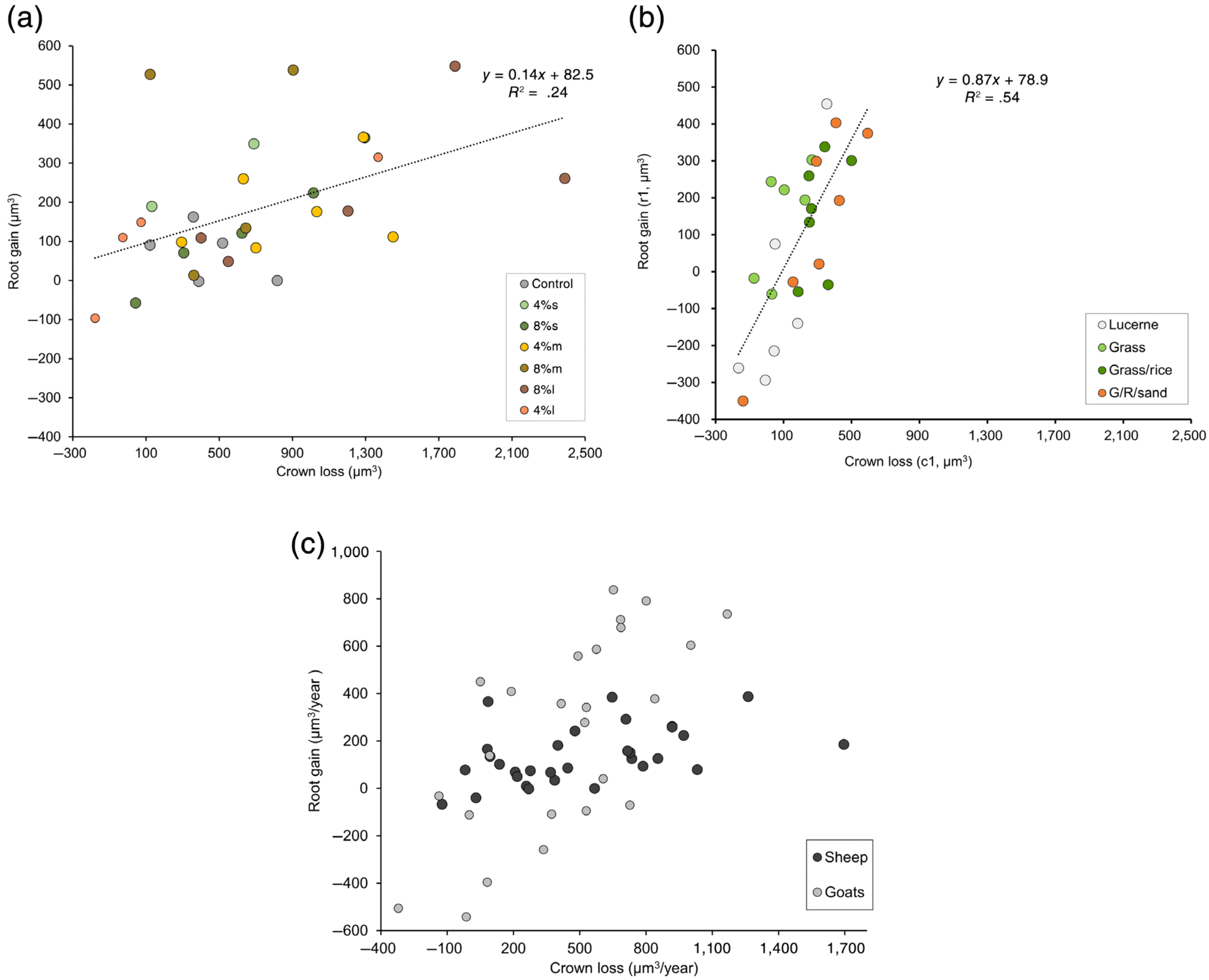

FIGURE 4 Correlation of crown loss and root gain on the right maxillary M2 for sheep ((a) *Ovis aries*, *n* = 31) and goats ((b) *Capra aegagrus hircus*, *n* = 26, adapted from Ackermans, Clauss, et al. (2019)) fed diets of varying abrasiveness for 17 and 6 months respectively. The experimental diets (a) include a control diet without added abrasives, two diets containing small abrasives (ø 4 μm) at two different concentrations 4%s and 8%s, and the same with both the medium and large abrasives (ø 50 μm, and 130 μm). (c) Yearly wear rate in sheep and goats plotted together

TABLE 3 Correlations between measurements on teeth of sheep (*Ovis aries*) fed diets of varying abrasiveness for 17 months

y–*x*	*n*	*R*	*p*
Root volume gain–crown volume loss	31	.49	**.005**
Burr mark growth–burr mark wear	18	−.23	.345[a]
Step height–tooth height	38	.47	**.003**
Valley depth–tooth height	38	.26	.119
Valley depth–step height	38	.44	.006[a]

Note: Correlations based on Pearson's test or Spearman's test (if data were not normally distributed). See Table 1 and Figure 1 for a definition of measurements. Bold values are statistically significant.

[a] Spearman's test.

David, 2006), differentiate into cementoblasts and secrete cementum (Berkovitz & Shellis, 2018). In spite of this difference, the feedback mechanisms that control the system should be of a similar or the same nature and must be linked to mechanoproprioception that reacts to pressure, in particular the lack of the pressure induced by contact with an antagonist tooth.

After feeding sheep experimental diets of varying abrasiveness for 17 months, volume loss in the crown segment was significantly correlated to volume gain in the root segment, though there was visually no distinction of different wear levels between diets. Compared to the pilot experiment on goats (Figure 4b), sheep appeared to have a higher volume loss in the crown segment and less root segment volume gain (Figure 4a,b). In the crown segment,

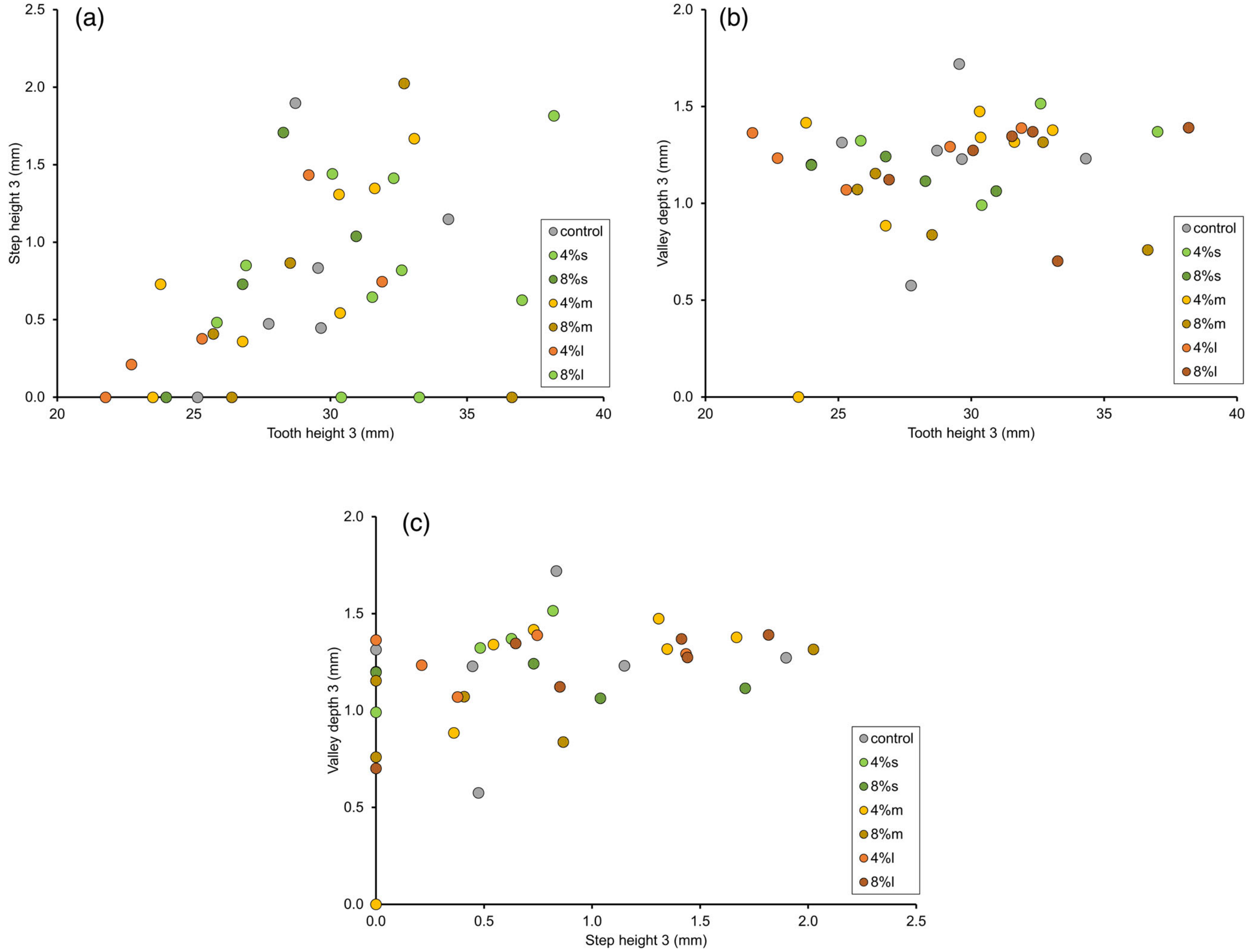

FIGURE 5 Correlations between tooth height, step height, and valley depth, measured on teeth of sheep (*Ovis aries*) fed diets of varying abrasiveness for 17 months. The experimental diets include a control diet without added abrasives, two diets containing small abrasives (⌀ 4 μm) at two different concentrations 4%s and 8%s, and the same with both the medium and large abrasives (⌀ 50 μm, and 130 μm). (a) Tooth height at the end of the experiment plotted against the height of the step between molar cusps at the end of the experiment. (b) Tooth height at the end of the experiment plotted against the depth of the valleys (dentine basins) at the end of the experiment. (c) Height of the step between molar cusps at the end of the experiment plotted against the depth of the valleys (dentine basins) at the end of the experiment

this effect could be explained by the substantially longer experimental period, while the relative lack of gain in the root segment may be attributed to the difference in diets. Notably, the experimental abrasives were much larger in the goats' diets (mean ⌀ 230 μm in the GRS diet, vs. mean ⌀ 4–130 μm in the sheep), and three of the four goat diets contained phytoliths, which may have created a larger effect of proprioception triggering an increase in cementogenesis. When calculating the measurements as wear rates (e.g., on a yearly basis), there was substantial overlap between the two species, although the relationship between crown segment loss and root segment gain did not appear as stringent and steep in the sheep compared to the goats (Figure 4c). Cementum growth has previously been recorded to be influenced by sex, higher rates having been found in female buffalo (Sanson et al., 2017), but sex cannot explain the differences here, as all animals were female (except one wether).

Tooth height loss in the sheep was ca. 1.78 mm/year as measured from burr mark to crown, but 1.29 mm/year when total tooth height was measured (Table 2), with the difference being most likely due to of the increase in volume of the root segment. Thus, the two measures seem to roughly corroborate each other. In comparison, a review of wear rates in mammals indicates less molar wear in other studies, for example, at 0.21–0.24 mm/year in the Iberian ibex (*Capra pyrenaica*), a mixed feeder, with the higher wear in a population that consumed a higher percentage of grass (Fandos, Orueta, & Aranda, 1993). Solounias et al. (1994) reviewed wear rates in mixed feeders at 0.9–1.6 mm/year, and of grazers at 2.0–3.7 mm/year, and Witzel et al. (2018) reported molar wear rates in Soay sheep on pasture at 2.7–3.1 mm/year. Therefore, the values found in our experimental sheep (and, by extrapolation from Figure 4c, also the goats) experienced wear in a range that would be expected for mixed feeders. Notably, this wear rate was not matched by distinct changes in mesowear scores in the goats (Ackermans et al., 2018). Compared to the fecal silica content of other wild herbivores (Hummel et al., 2011), neither the feces of the goats (Ackermans et al., 2018) nor sheep (personal observation) showed particularly high silica levels, suggesting that the levels of experimental abrasives were not excessive compared to natural situations. Rather, there is the possibility that free-ranging animals ingest higher amounts of soil when foraging freely, as well as having a higher food intake than our experimental animals due to higher levels of activity, also but not exclusively due to reproduction. This would translate into higher wear rates, as in the Soay sheep at pasture.

In principle, our absolute wear measurements, either through volume reconstruction, or a digital caliper with or without burr marks, record tooth height loss as expected. The expectations for a gain in root segment volume were met, but root length measurements using the burr mark (measuring either the distance to the alveolar margin or to the root apex) were not significant, even though they showed a numerical increase (Table 1). The resulting impression is that these measurements have potential for investigating general principles of tooth wear and morphological change, but are comparatively crude and are not suited to detect more subtle dietary differences in these animals.

The sensitivity of volumetric measurement is tightly related to CT scan quality (Figure 2). Micro-CT scans would have been ideal for these types of absolute wear volume measurements (comparing Figure 2c,d), but to date it is not yet possible to perform micro-CT scans on live animals. Secondly, marking the teeth with burrs is certainly durable, but tooth wear, chipping, and CT visualization mitigate their persistence (Figure 2a,b). The low number of specimens with measurable burr marks was likely an important factor in some of the discrepancies between measurements related to this parameter. Although animals were all selected to be of a similar wear stage, excluding animals with overly worn teeth, the variation in age of the experimental sheep is a factor to be considered, as wear can differ depending on an animal's age, especially in the case of very young or old animals (Rivals, Mihlbachler, & Solounias, 2007). Future studies should strive to ascertain the age of the individual sheep with more accuracy than what was possible for us, ideally raising a flock of same year-lambs until maturity for an experiment. Finally, the crude denomination of a "crown segment" and a "root segment" was used here for simplicity, even though enamel can extend past the infundibulum into the roots, exposed dentine can be found on the, occlusal surface and mesially (Witzel et al., 2018), and cementum can be deposited on the crown, notably in bovids (Ainamo, 1970). This delimitation was purely selected as a linear way to separate the tooth segment actively gaining volume, from the segment losing volume. In the pilot experiment, different ways of achieving the dichotomic separation of molars in CT scans had been tested, leaving the method adopted here as the one with the most distinct signal (Ackermans, Clauss, et al., 2019).

Digital measurements revealed some correlations between different anatomical features within the tooth. Namely, higher teeth generally also had higher steps at the infundibulum, suggesting that in the process of wear, the molar surface is generally flattened. Nevertheless, some teeth did not have a measurable step, even though they were not among the most worn (Figure 5a). There was no change in the dentine basin, or valley depth over time, matching a similar observation on wild buffalo (Sanson et al., 2017). Martin et al. (2019) suggested that in animals with hypsodont (as opposed to ever-growing, hypselodont) teeth, there is a rather constant equilibrium, where dentine erosion exposes the enamel, contributing to enamel wear, with a constant enamel–dentine ratio. This is also suggested in our results, by the consistency of the valley depth measurement (Figure 5b).

The reaction of periodontal ligaments to tooth displacement have been investigated in rats (Naveh, Shahar, Brumfeld, & Weiner, 2012; Niver et al., 2011), including using micro-CT to target high-tension zones prone to more mineral formation (Lin et al., 2013). However, the link to crown volume loss, as well as to cellular proliferation, is missing. Collaboration between tooth physiology and tooth wear research groups is warranted to better understand these phenomena, especially on the case of hypsodont teeth.

5 | CONCLUSIONS

The correlation between volume loss in the crown segment and volume gain in the root segment, observed first in the pilot study, and again in the present experimental study, anchors the existence of a feedback mechanism triggering root growth as a form of compensation for crown wear. As the understanding of this mechanism is rudimental at best, work remains in exploring its extent across the dentition of hypsodont animals and their various diets. For example, the effect abrasive diets create for root growth remains to be investigated in nonruminant ungulates. Though we expect cementum to be the main factor causing the volumetric increase in the root area, we cannot say for certain that this is the case without more precise imaging techniques than applied in the present study. The biomechanisms connecting dietary properties, such as abrasion and hardness, to cell proliferation—and thus root growth—remain poorly understood. Further experiments are required to illuminate the underlying mechanism, including the participation of each of the dental layers, whether a precise loading point triggers the tooth's proprioceptors, and how this mechanism reacts to external versus internal abrasives from herbivore diets.

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CONFLICT OF INTEREST

The authors declare no potential conflict of interest.

AUTHOR CONTRIBUTIONS

Jean-Michel Hatt and Marcus Clauss designed the study; Nicole L. Ackermans, Louise F. Martin, Marcus Clauss, and Jean-Michel Hatt performed the experiment; Partick R. Kircher supervised the CT scanning; Nicole L. Ackermans performed the volumetric and caliper measurements; Daryl Codron analyzed the data, Nicole L. Ackermans and Marcus Clauss wrote the first draft of the manuscript, which then received input from all coauthors. All authors give their final approval and agree to be accountable for all aspects of the work.

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SUPPORTING INFORMATION

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